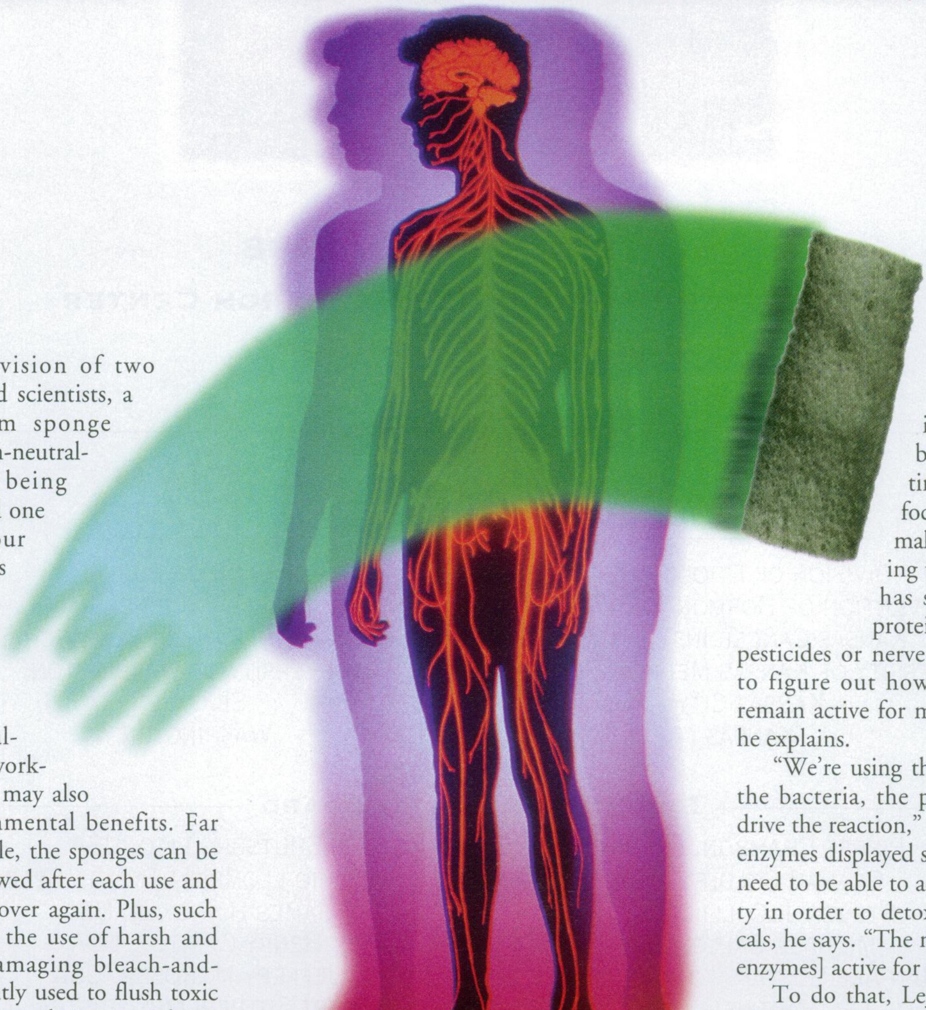
# Super Sponges



Thanks to the vision of two Pittsburgh-based scientists, a polyurethane foam sponge incorporating a toxin-neutralizing enzyme is being developed that could one day be used to scour harmful nerve agents from the skin and clothing of soldiers on the battlefield, as well as harmful pesticides from that of agricultural or industrial workers. Their invention may also boast some environmental benefits. Far from being disposable, the sponges can be reactivated and renewed after each use and employed over and over again. Plus, such sponges might trim the use of harsh and environmentally damaging bleach-and-water systems currently used to flush toxic nerve agents from exposed victims and surfaces.

To destroy the deadly nerve agents in use after use, however, the enzyme itself must remain active inside the sponge structure. Keith E. LeJeune, a Carnegie Mellon University graduate student, and Alan J. Russell, a chemical engineering professor at the University of Pittsburgh, have managed to immobilize the enzyme phosphotriesterase in a matrix of polyurethane foam, and in doing so, have boosted the enzyme's utility. Tethered firmly to the polyurethane foam matrix through covalent links, the enzyme becomes stable at room temperature. Thus, explains LeJeune, it retains a high level of activity over a long period of time.

"The idea of stabilizing proteins is nothing new; people have been doing it for a long time," says Russell. "Our focus is on doing it while making polymers." According to Russell, no one so far has significantly stabilized proteins capable of degrading pesticides or nerve agents. "Our goal was to figure out how to make the enzyme remain active for much more than a day," he explains.

"We're using the cellular machinery of the bacteria, the proteins themselves, to drive the reaction," says LeJeune. While the enzymes displayed short-term stability, they need to be able to achieve long-term stability in order to detoxify the harmful chemicals, he says. "The main goal is to [keep the enzymes] active for a long period of time."

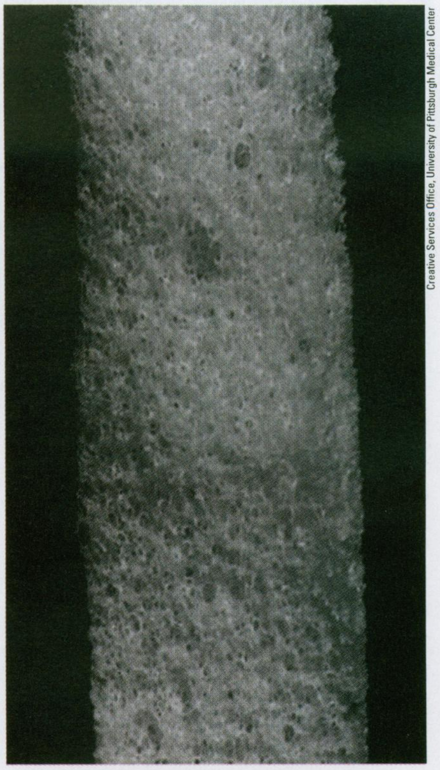
To do that, LeJeune linked the phosphotriesterase covalently to the foam matrix. The enzyme remains active and hydrolyzes the organophosphate bond in the pesticide paraoxon. Experts already know that phosphotriesterase can detoxify some nerve agents used in chemical warfare, such as sarin. In their work, LeJeune and Russell have determined that a mere 2.5 kg of immobilized phosphotriesterase could detoxify 30,000 tons of nerve agent over a one-year period.

The United States Army, which is funding Russell's and LeJeune's basic research through the Army Research Office, has found their results so promising that researchers at several other Army laboratories—including the Walter Reed Army Institute of Research in Washington, DC, the Natick Research, Development and Engineering Center in Massachusetts, the Edgewood Research, Development and Engineering Center in Maryland, and the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) in Aberdeen, Maryland—are now collaborating with the Pittsburgh-based team.

At the request of Bhupendra P. Doctor, director of Walter Reed's division of biochemistry, LeJeune created polyurethane sponges incorporating two enzymes—acetylcholinesterase (AChE), which influences nerve-to-nerve transmissions in humans, and butyrylcholinesterase (BuChE). Both of these enzymes are targets of organophosphorous agents (OPs). Using these sponges, Doctor and colleague Richard K. Gordon, a research chemist at Walter Reed, have shown in a battery of *in vitro* tests that the enzymes indeed link very efficiently to the polymer (sponge) matrix, and that, upon doing so, they stay active. Stored dry at 4°C, the enzyme sponges remain stable for a minimum of three years without losing activity. Doctor and Gordon also suggest that the sponges may be suitable for desert environments, where, despite exposure to damagingly high temperatures, the enzymes may lose only half of their activity within a day's time—encouraging news since U.S. forces could be deployed once more to the Persian Gulf.

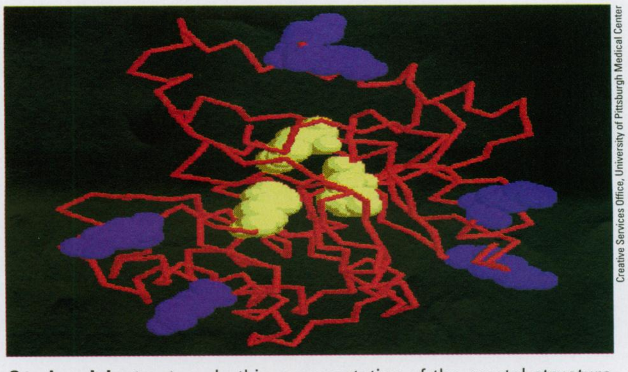
Also encouraging is the fact that the sponges, once used, can be revived. According to Doctor, the enzymes in the sponge can be reactivated by dipping the sponge into a buffered oxime solution, yielding only a tiny loss of enzyme after many reactivations. Doctor expects the sponges to be field-tested in 2–3 years.

Doctor and colleague Michelle Ross, acting chief of the drug assessment division of the USAMRICD, are currently writing animal protocols to test the sponges for decontamination of the skin of rats or nude guinea pigs exposed to toxic organophosphates. "If it looks promising, we'll be able to contribute substantially to solving this problem," contends Doctor, who has been working for the past decade on related research. "For the past 10–12 years our group has been working on verifying the idea that you could use cholinesterase to scavenge and detoxify organophosphate nerve agents," he says.




**Holey moly!** Enzyme-infused sponges are being developed in several different porosities to suit a range of uses.

Doctor is collaborating with another researcher, David E. Lenz, chief of the biochemical pharmacology branch of the USAMRICD, who will subject the enzyme-infused sponges to sarin and soman, both G-series nerve agents. Once the enzyme foam system proves safe and effective in laboratory animals—a process that should be complete within the next two years—the researchers will head straight to the Food and Drug Administration for approval to develop this material for use on humans.




**Scrub-a-dub structure.** In this representation of the crystal structure for organophosphorus hydrolase, the blue color indicates surface lysine amino acid residues, which can participate in the polymerization reaction. The yellow residues represent the enzyme's active site.

Lenz's lab has yielded another promising development—a mutated form of BuChE that, according to Lenz, is not inhibited by organophosphorous nerve agents, and that actually catalyzes the hydrolysis of the OPs. And, unlike its nonmutant cousin, this mutated enzyme needn't be reactivated in an oxime solution. Still, the mutated BuChE cannot hydrolyze the nerve agents at a fast enough rate to provide adequate protection for humans, which makes this version of the enzyme foam system years away from being realized.

## Many Forms, Many Uses

The enzyme foam detoxification system could take many forms, says Doctor. "The sponge is something we started out with. It could take any form or shape that we could polymerize—a towelette or a cotton swab," he says, citing two examples. And, according to Army researchers, the polyurethane-immobilized enzyme could be formed into granules, then put into a topical cream. Once applied to human skin, the cream could detoxify nerve agents. The enzyme may also be incorporated into fabrics for use in protective garments. Such a fabric, if proven effective as a detoxifier, would weigh far less than the three-layer protective suit currently worn by soldiers, which adsorbs toxic agents via a middle layer of activated carbon in polyurethane foam.

Researchers working at the Natick Research, Development and Engineering Center have been testing the foam pioneered by LeJeune and Russell, and are interested in materials that block toxic vapors. During the past year or so, their tests have confirmed that the phosphotriesterase sponge is indeed effective against the pesticide paraoxon when it is introduced in liquid form, though the vapor tests done so far haven't yielded sufficient conversion levels. Nevertheless, the search for the right fabric continues. Heidi L. Gibson, a polymer researcher at the center, is tapping electrical current to create long, ultrathin fibers that are 10–100 times finer than typical textile fibers in a process called electrospinning. She aims to incorporate active enzymes into these nanofibers, which ideally would be woven into a comfortable, lightweight, toxin-killing fabric for protective clothing.

Another application, according to Russell, would be fire-fighting foam, a product with a texture he likens to shaving cream. "There's always been this problem of what to do with a large pesticide spill," he says. "How do you treat a wide area of contamination? How do you treat a field or a building?" Russell and his colleagues have submitted a paper for publication on their fire-fighting foam research.

LeJeune is currently performing tests to see if phosphotriesterase holds its own and remains stable in such a foam. If the enzyme does remain active, LeJeune hopes to go back and immobilize it in polyurethane, then grind up the resulting polymer into a fine powder that would go into a recipe for the fire-fighting foam. This enzyme-polymer powder may also replace the bleach—or at least part of it—found in German emulsion, LeJeune claims. German emulsion is simply a mixture of bleach, water, and an organic solvent, which together detoxify nerve agents. According to LeJeune, replacing all or part of the bleach with the powder would reduce the negative environmental impact of German emulsion.

Researchers at the Edgewood Research, Development and Engineering Center share Russell's and LeJeune's interest in a liquid-based enzyme foam. Under the direction of Joseph J. DeFrank, team leader for environmental technology, researchers there are hard at work on enzyme-containing solutions that could be sprayed as a liquid into a foam that might decontaminate large areas such as airports, seaports, battlefields, and civilian properties. In addition, DeFrank thinks the enzyme-laden sponges could be deployed to wipe down small surfaces or sensitive electronic equipment, like that found in aircraft cockpits.

The nerve agents used in chemical warfare and terrorist situations are simply stronger versions of agricultural pesticides, and share the same mechanisms in the human body, says LeJeune. Methyl parathion, for example, is used by the U.S. cotton industry to control boll weevils. In South America and Africa, it is used in a variety of agricultural applications, according to LeJeune. In 1996, methyl parathion, which attacks the central nervous system, was inappropriately used for household pest control in homes in Mississippi. The detoxifying sponges might be used in such an emergency instance, says LeJeune, if the need were to arise.

### Biology-driven Solutions

Eventually, LeJeune thinks, some form of enzyme-driven bioremediation could be used to safely decontaminate pesticide-infested wastewater in agricultural settings. And, because the enzyme–polyurethane product both adsorbs and breaks down organic contaminants, it could also be used in fume hood filters to improve air quality in industrial settings as well. "Such foams could potentially be the last stop in an air-cleaning process," says LeJeune.

Ultimately, any number of different proteins could be put into polyurethane foam, says Russell, each with the potential to target different toxins. "The project we're doing now is to incorporate multiple enzymes into the same foam to attack multiple agents," he explains.

Among the challenges facing the researchers in their quest to bring this enzyme foam technology to market are availability of the enzymes, as well as determining which enzymes attack which chemical agents. Cost itself doesn't pose a concern, say both LeJeune and Russell. "The enzyme is very active, so you don't need much," explains Russell. "It's not costly to produce if there is adequate demand."

And, adds Doctor, once researchers find the right enzymes to solve a particular toxic ill, enzymes could be bioengineered and produced in large quantities, and thus be readily available to the marketplace.

Indeed, while the U.S. military awaits the technology to protect soldiers from the ravages of chemical weapons on the battlefield, these same scientific efforts may one day lead to novel remedies for civilian workers in industry and agriculture who are subjected to harmful chemicals on the job.

**Jennifer F. Medlin**

## Suggested Reading

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